Pestic. Sci. 1997, **50**, 221–227

Biological Activity of Two Stereoisomers of the *N*-Thienyl Chloroacetamide Herbicide Dimethenamid

Michel Couderchet,[1,2]* Pierre F. Bocion,[3] Reynold Chollet,[3] Karl Seckinger[4] & Peter Böger[2]

[1] Lab. Zool. & Sci. Environnement, UFR Sciences, Université de Reims Champagne Ardenne, BP 1039, F-51687 Reims-Cédex 2, France
[2] Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-78434 Konstanz, Germany
[3] Agrobiologische Versuchsstation, Sandoz Agro AG, CH-4108 Witterswil, Switzerland
[4] Sandoz Agro AG, CH-4002 Basel, Switzerland

(Received 30 August 1996; revised version received 13 December 1996; accepted 28 February 1997)

Abstract: Due to the presence of an asymmetrically substituted C atom, dimethenamid [2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide], a recently introduced *N*-thienyl chloroacetamide herbicide, exists as two stereoisomers (*S* and *R*) having differing herbicidal activities as demonstrated with a selection of weeds and *Lemna minor*. The activity of the two isomers was investigated in greater detail with the green alga *Scenedesmus acutus* and compared to that of alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide]. As with alachlor, the *S* isomer (5 μM) strongly inhibited algal growth and fatty acid desaturation while the *R* isomer had no effect. In short-term experiments (up to 5·5 h), the *S* isomer and alachlor (100 μM) inhibited [^{14}C]acetate uptake and its incorporation into fatty acids in the same manner, while the *R* isomer did not. Incorporation of [^{14}C]acetate into a non-lipid fraction of the algae was strongly inhibited by alachlor and the *S* isomer (100 μM) and only slightly by the *R* isomer. A 50% inhibition of incorporation of [^{14}C]oleic acid into the same non-lipid fraction was attained with less than 10^{-7} M of the *S* isomer while 10^{-5} M of the *R* form of dimethenamid achieved only a 40% inhibition. The same stereospecificity of the compound on growth, fatty acid desaturation, acetate uptake and oleic acid incorporation provides strong evidence that dimethenamid may act upon a primary, specific target in lipid metabolism. Furthermore, the comparable biological activities of dimethenamid and alachlor indicate that this target is common to both *N*-phenyl and *N*-thienyl chloroacetamide herbicides.

Pestic Sci., **50**, 221–227, 1997
No. of Figures: 7. No. of Tables: 0. No. of Refs: 23

Key words: enantiomer, fatty acid, Frontier, dimethenamid, chloroacetamide, *Scenedesmus acutus*

* To whom correspondence should be addressed.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

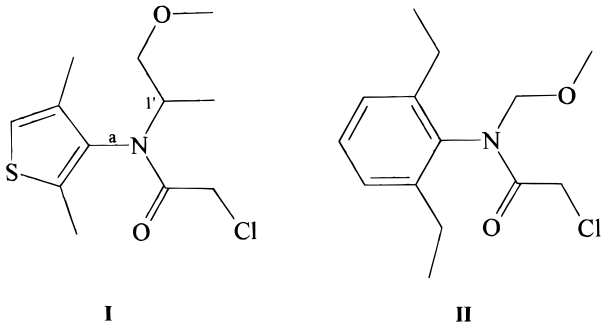
1 INTRODUCTION

Dimethenamid (2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide; Fig. 1, **I**) is a recently introduced pre-emergence *N*-thienyl chloroacetamide herbicide for the control of annual grasses and some broadleaf weeds in maize and soybean.[1] When applied pre-emergence at shoot level, relatively low rates are sufficient to totally control certain weeds (100 g AI ha^{-1} and 25 g AI ha^{-1} for *Echinochloa crus-galli* (L.) Beauv. and *Setaria viridis* (L.) Beauv., respectively) whereas crops such as maize and soybean can tolerate rates of several kg AI ha^{-1}.[2]

The presence in the molecule of a chiral axis and an asymmetrically substituted carbon atom (Fig. 1) yields four stereoisomers a*S*,1′*S;* a*R*,1′*S*; a*S*,1′*R* and a*R*,1′*R*. No major difference in the biological activity of a*R* and a*S* isomers has been found.[3] Furthermore, the low energy required for the rotation around the chiral axis results in racemisation and two main isomers can be distinguished: a*RS*,1′*S* (abbreviated to *S*) and a*RS*,1′*R* (or *R*); the racemate is a*RS*,1′*RS* (or *RS*). The *S* form has been shown to be much more active than the *R* form on different organisms.[1,3] The biochemical reason for this difference of activity between the two isomers remains to be investigated.

The structural similarity between *N*-thienyl and *N*-phenyl chloroacetamide herbicides and their weed-control spectra suggests a common biochemical mode of action for the two herbicide classes. Although the primary mode of action of the chloroacetamide herbicides is not known, many effects have been observed.[4,5] In the green alga *Scenedesmus acutus*, metazachlor and alachlor inhibited fatty acid desaturation,[6,7] uptake of acetate by the cells[8] and its incorporation into a non-lipid fraction.[9] More recently a 50% inhibition of oleic acid incorporation into a non-lipid fraction of *Scenedesmus acutus* was found with a concentration of metazachlor lower than 10^{-7} M,[7] this inhibition was not observed in a metazachlor-resistant cell line of the alga[10] and seemed specific to acetamide herbicides.[11]

In this study, the biological activity of the two main stereoisomers of dimethenamid (*R* and *S*) was investigated in whole plants treated pre-emergence, in *Lemna minor* L. and in more detail in *S. acutus*. In all assays with the green alga, *R* and *S* were compared with alachlor, a chloroacetamide herbicide with no chiral axis or asymmetrically substituted carbon atom (Fig. 1, **II**).



I II

Fig. 1. Structures of dimethenamid (**I**; a: chiral axis, 1′: asymmetrically substituted C atom) and alachlor (**II**).

2 MATERIALS AND METHODS

2.1 Plant material

Eight grasses (*Apera spica-venti* (L.) Beauv., *Bromus tectorum* L., *Digitaria sanguinalis* (L.) Scop., *E. crus-galli*, *Eleusine indica* (L.) Gaertn., *Lolium perenne* L., *Poa annua* L. and *Setaria faberi* Herrm.) and five broadleaf weeds (*Amaranthus retroflexus* L., *Datura stramonium* L., *Gallium aparine* L., *Portulaca oleracea* L. and *Senecio vulgaris* L.) were grown in the greenhouse under normal daylight conditions.

The duckweed *L. minor* was grown in a mineral medium as described.[12]

Sterile liquid cultures of the green alga *S. acutus* no. 276-3a (Algae Collection, University of Göttingen, Germany) were grown as described.[13] The algae were inoculated with a packed cell volume (pcv) of 0·3 $\mu l\ ml^{-1}$ except for incorporation studies in which the pcv at inoculation was approximately 2 $\mu l\ ml^{-1}$.

2.2 Herbicides

Alachlor (99·4% pure) was purchased from Dr Ehrenstorfer (Augsburg, Germany). The two isomers of dimethenamid (*R* and *S*) were 99·4% pure (GC analysis); the enantiomeric purity was greater than 98%. For experiments using *S. acutus*, stock solutions of the herbicides were made in ethanol so that the maximum final ethanol concentration was 1 ml $litre^{-1}$ in short-term experiments (up to 5·5 h) and 0·05 ml $litre^{-1}$ in incubation over 24 h. At these concentrations ethanol had no effect on the algae, and its concentration was the same in the controls and herbicide-treated algae.

2.3 Higher plant herbicide treatment and evaluation of activity

The weeds were treated pre-emergence at 28, 83, 250 and 750 g AI ha^{-1} with a spray volume of 600 litre ha^{-1}. The two isomers were formulated (250 g kg^{-1} emulsifiable concentrate) and applied in a spraying chamber. The herbicidal activity was assessed visually after four weeks (0 = no effect, 100 = total kill) and expressed as an average value for the 13 tested species.

Frond pairs of *L. minor* were treated by dissolving the herbicide in the medium. Treatments were rated after six days, and growth inhibition was expressed as a percentage of the new fronds grown in the untreated control.[12]

2.4 Fatty acid extraction and determination

After incubation of *S. acutus* for 41 and 68 h with or without herbicide, fatty acids were extracted and methylated as described earlier.[13] Unlabelled fatty acid methyl esters (0·1 μl in hexane) were separated using a gas chromatograph (Perkin-Elmer F22, Uberlingen, Germany) equipped with a flame ionisation detector (FID). The carrier gas (nitrogen) supply was 3 ml min^{-1}. The injector and detector temperature was 250°C and that of the oven was 165°C. Separation was performed using a fused silica capillary column (SP-2330, 30 m × 0·25 mm, Supelco, Bad Homburg, Germany). Identification was based on comparison with reference methyl esters for the most common fatty acids. C16 : 2 to C16 : 4 and C18 : 4 were identified by mass spectrometry, and after separation according to the degree of unsaturation by solid phase extraction with silver-nitrate-impregnated Bond Elut SCX columns (Analytichem International, ICT, Frankfurt, Germany) as described earlier.[7] (In the nomenclature used for the fatty acids, Cx : y, x represents the number of carbon atoms and y the number of double bonds.)

2.5 [^{14}C]Acetate incorporation

A one-day-old culture of *S. acutus* (approximately 20 μg chlorophyll ml^{-1} and 4 μl pcv ml^{-1}) was concentrated to 50 μg chlorophyll ml^{-1}. Ethanol solution of herbicide (Section 2.2) was added to 10 ml of the algae suspension together with 20 μmol of non-labelled sodium acetate. The algae were incubated for 30 min in the light (80 μE m^{-2} s^{-1}, provided by fluorescent lamps) at a temperature of 21°C, and air supplemented with carbon dioxide (20 ml $litre^{-1}$) was bubbled through the vessel. After this short incubation [2-^{14}C]acetate (3 nmol, specific activity 1·96 TBq mol^{-1}, approximately 5880 Bq; Amersham, Braunschweig, Germany) was added to the sample and incubation proceeded under the same conditions. At one- to two-hour intervals, 1 ml of the algal suspension was collected, centrifuged and the label remaining in the incubation medium counted as a measure of acetate uptake. The pellet was further investigated as follows.

2.6 [^{14}C]Oleic acid incorporation

A one-day culture of *S. acutus* (approximately 20 μg chlorophyll ml^{-1} and 4 μl pcv ml^{-1}) was concentrated to 100 μg chlorophyll ml^{-1}. [1-^{14}C]Oleic acid (2·65 nmol, specific activity 1·95 TBq mol^{-1}; Amersham, Braunschweig, Germany) dissolved in toluene was placed into a 10-ml incubation vessel, dried under nitrogen, 5 ml of algal growth medium was added and the oleic acid dispersed by ultrasonication for 5 min. Five millilitres of the concentrated algal culture, containing herbicide or not, was added to the oleic-acid-containing medium to reach a concentration of 50 μg chlorophyll ml^{-1}. The samples were then incubated under conditions identical to those described for acetate incorporation.

2.7 Incorporation of radioactivity into fatty acids and into the non-lipid fraction

The algal cells were centrifuged (5 min, *c.*6000*g*), washed and resuspended in 1 ml water. Methanolic potassium hydroxide (10 g $litre^{-1}$, 5 ml) was added, the tube capped and incubated at 65°C for 90 min to allow for total saponification of the lipids, during which a precipitate was formed. The sample was cooled, centrifuged (5 min, *c.*6000*g*) and the supernatant was decanted into another tube, the fatty acids extracted, methylated and counted for radioactivity. The pellet which represented the 'saponification precipitate' (compare Refs 7, 9), was washed with hexane (2 × 5 ml) and methanol (2 × 5 ml). The resulting non-lipid fraction was dissolved in water (1 ml) and counted for radioactivity.

Radioactivity was quantified by liquid scintillation (LKB 1215, Rack Beta II, Pharmacia, Freiburg, Germany) after mixing an aliquot of the radioactive sample with scintillation cocktail (Insta Gel II, Packard, Frankfurt, Germany).

2.8 Statistics

The experiments were repeated at least twice. The most representative experiments are shown and deviation from the data presented was never more than 10%.

3 RESULTS AND DISCUSSION

3.1 Activity in higher plants

Overall, the weed species examined were much more sensitive to the *S* form than to the *R* form. Indeed, the dose rates needed to give a particular level of damage were approximately 30 times lower with the *S* isomer than with the *R* isomer (Fig. 2). Although some differences in sensitivity exist among the 13 weeds tested, they were all well controlled by dimethenamid and their behaviour with regard to the two isomers was similar. The difference in activity of the stereoisomers was even more pronounced with *L. minor*, in which the *S* form was approximately 100 times more active than the *R* form (Fig. 3). *L. minor* may be much more sensitive to the *S* isomer than the conventional weed species tested, while the *R* form would be almost inactive on both types of plant. The higher sensitivity of *Lemna* comes from a permanent contact with the herbicide dissolved in the nutrient solution, a thinner cuticle (especially on the lower side of the fronds), and a shorter distance for the herbicide to reach its biochemical target (no stem, no petiole) meaning less time for detoxification. The

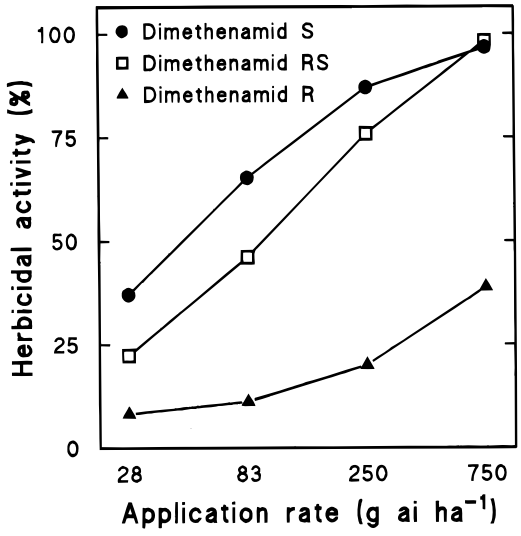


Fig. 2. Influence of dimethenamid and of its two isomers after pre-emergence application. The herbicidal activity was assessed visually (0 = no effect, 100 = total kill) and expressed as average value for eight grasses and five broadleaf weeds (for species see Section 2.1).

activity of the *R* form may be due to the presence of traces of the *S* form (1% *S* form in the *R* form would be sufficient for such an activity).

3.2 Growth and fatty acid profile of *Scenedesmus acutus*

As measured with packed cell volume, growth of the culture was dramatically reduced by 5 μM of the *S* form of dimethenamid during the first 40 h and almost stopped thereafter (Fig. 4). The effect of this isomer was similar to that of alachlor. At this concentration the *R* isomer had almost no effect on growth.

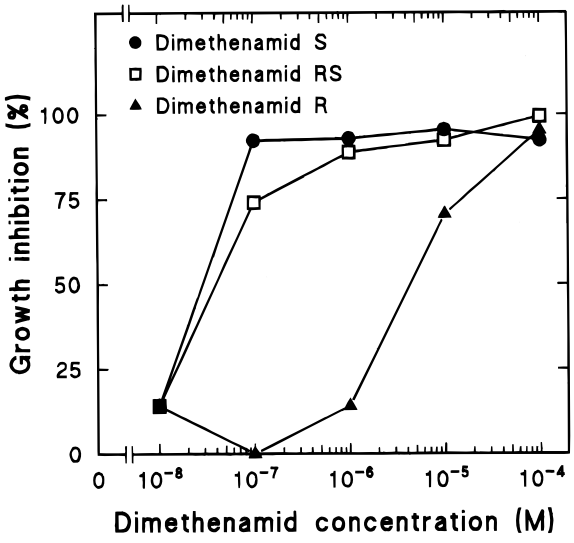


Fig. 3. Influence of dimethenamid and of its two isomers on the growth of *Lemna minor*. Assessments were performed by counting after six days of treatment, and growth inhibition expressed in percentage of the new fronds grown in the untreated control.

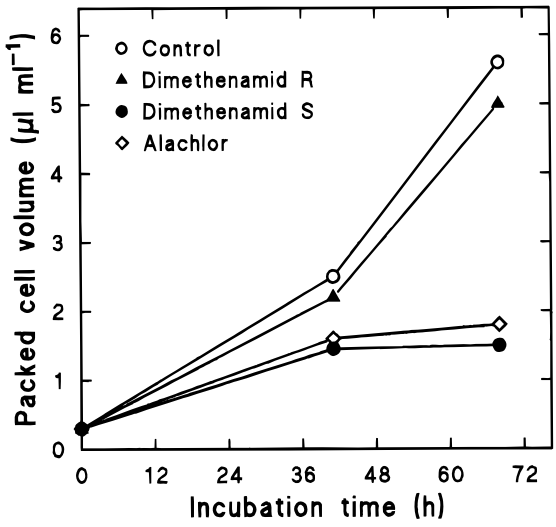


Fig. 4. Influence of two isomers of dimethenamid and of alachlor on growth of *Scenedesmus acutus* in liquid culture. At the beginning of incubation, the packed cell volume was 0·3 μl ml^{-1} and herbicide concentration 5 μM (*R* = a*RS*,1′*R*; *S* = a*RS*,1′*S*).

The fatty acids most abundant in *S. acutus* are C18 : 3, C16 : 0, C16 : 4 and C18 : 2; all the other fatty acids were found to represent less than 5% each (Fig. 5A and B, control). This is consistent with the fatty acid composition previously reported by our laboratory for this alga.[7]

Fatty acid desaturation was strongly decreased by 5 μM of the *S* isomer and alachlor (Fig. 5A), the relative amounts of C16 : 4, C18 : 2, C18 : 3 and C18 : 4 decreasing while those of C18 : 1, C18 : 0 and C16 : 0 increased. This effect was even more pronounced after a longer incubation time (Fig. 5B), when all polyenoic fatty acids were less than in the control, and it was also comparable to that of metazachlor.[7] In this respect again algae treated with the *R* form of the herbicide behaved similarly to the controls.

3.3 Acetate uptake and incorporation into fatty acids and the non-lipid fraction

Acetate was readily taken up by *S. acutus* control cells and after 3·5 h almost no radioactivity was left in the medium; uptake was not influenced by the *R* isomer (100 μM) of dimethenamid (Fig. 6A). As described earlier for metazachlor,[8] acetate uptake was lowered by both the *S* isomer (100 μM) and alachlor (100 μM). Inhibition was about 50% whilst acetate was still present in the medium, that is for 2·25 h. When acetate was no longer found in the medium of control cells (after 3·5 h) inhibition declined to approximately 25% and was no longer detectable after 5·5 h, when the treated cells had also consumed all the acetate in the medium. *S. acutus* cells growing autotrophically can use acetate as a carbon source when present in the medium.[14] This is consistent with the results presented in our study in which the cells

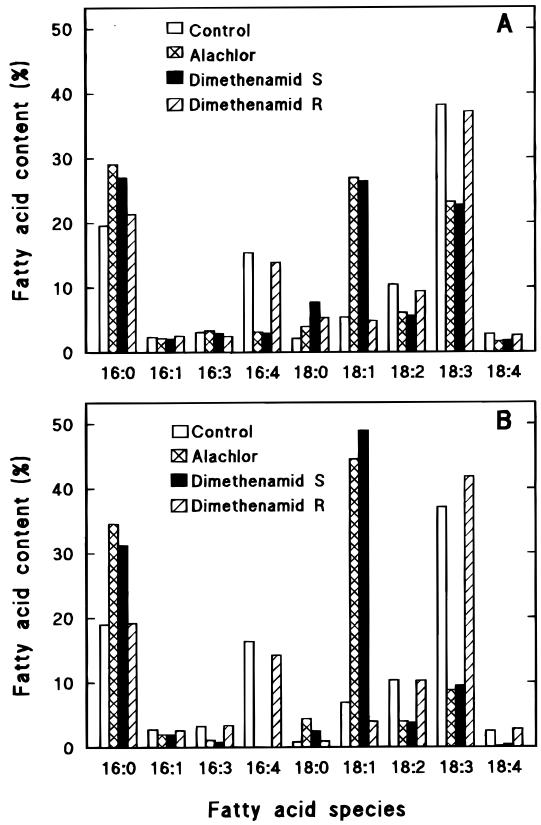


Fig. 5. Influence of two isomers of dimethenamid and of alachlor on the fatty acid profile of *Scenedesmus acutus* after (A) 41 h or (B) 68 h incubation. At the beginning of incubation, packed cell volume was 0·3 μl ml^{-1} and herbicide concentration 5 μM (R = aRS,1′R; S = aRS,1′S).

have taken up the 2 mM acetate present in their medium after 3·5 h.

Half of the labelled acetate taken up by the cells was recovered in the fatty acid fraction (Fig. 6B). Incorporation of the label in fatty acids was reduced by dimethenamid *S* and alachlor in a manner comparable to that of acetate uptake. When the label incorporated in fatty acids was expressed as percentage of acetate taken up by the cells, it fluctuated (between 45 and 55%) for all assay periods and herbicide treatments and no inhibition could be detected. This indicated that inhibition of fatty acid formation was a direct function of acetate uptake. It was not possible to identify whether the incorporation of the acetate label into fatty acids was lower because of a lower uptake or vice versa. Again, the *R* isomer had no measurable effect.

In the control cells the proportion of radioactivity incorporated into the non-lipid fraction (or saponification precipitate) varied from 4·6% of the total acetate taken up after 1 h incubation to 6·9% after 5·5 h (Fig. 6C). Inhibition by dimethenamid *S* and alachlor of incorporation of acetate into the non-lipid fraction was stronger than into fatty acids and could not solely be explained by uptake inhibition, since after 5·5 h radioactivity incorporated into that fraction was reduced from 6·9% in the control to 5·1, 3·0 and 2·5% when the

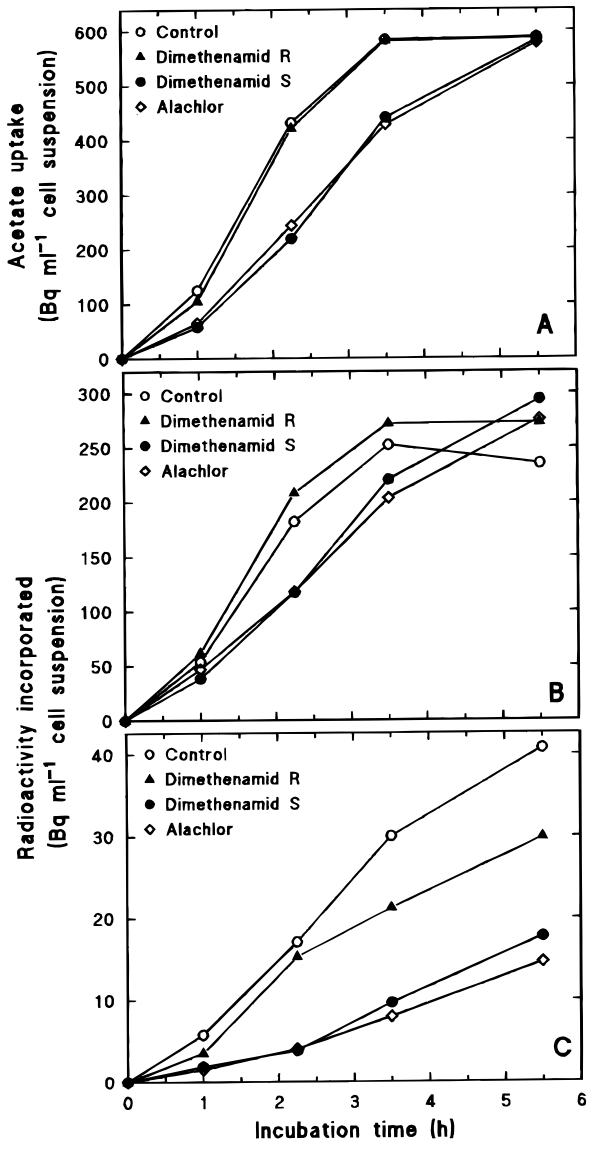


Fig. 6. Acetate uptake (A) by *Scenedesmus acutus* and its incorporation into (B) fatty acids and (C) a non-lipid fraction. After the culture (50 μg chlorophyll ml^{-1}) had equilibrated for 30 min with non-labelled acetate (2 mM) and herbicide (100 μM), [2-^{14}C]acetate (5880 Bq) was added to the 10 ml suspension.

algae were treated with dimethenamid *R*, *S* and alachlor, respectively. This effect of chloroacetamides was first described for alachlor and metazachlor in 1991.[9] It was then hypothesised that acetylation of DNA-associated proteins (possibly histones) could be inhibited by the herbicides. However, up to now we have not been able to demonstrate an *in-vitro* inhibition of histone acetylation; the isolated histone acetyltransferase of maize and barley could not be inhibited by alachlor or metazachlor.[15] Nevertheless, this effect of the chloroacetamides is a potent one and deserves further investigation.

Inhibition of acetate incorporation into the non-lipid fraction is also inhibited by the *R* isomer. After 5·5 h of incubation this inhibition was 27% but this is much

lower than that found for alachlor (64%) and the *S* isomer (56%). This inhibition by the *R* isomer is consistent with the fact that low herbicidal activity was detected at high concentrations in higher plants (Figs 2 and 3) and in *Scenedesmus*. In this alga, 100 μM of the *R* isomer inhibited growth of *Scenedesmus* by 50%, but this inhibition was attained with approximately 1 μM of the *S* form.[1] Since inhibition of acetate incorporation into the non-lipid fraction is a potent effect of the chloroacetamides, a weak inhibition of the *R* isomer could be expected. Contamination of the *R* isomer by traces of the *S* isomer is another possible explanation (see Section 3.1).

3.4 Incorporation of oleic acid in the non-lipid fraction

Oleic acid was also readily incorporated into the cells. After 1 h almost all radioactivity had disappeared from the incubation medium. However, the uptake of acetate and oleic acid cannot be compared because of the different concentrations in the medium (2 mM and 2·65 μM for acetate and oleic acid, respectively). Furthermore, care should be taken when interpreting uptake studies with oleic acid since it is not water-soluble and has been shown to adsorb onto glass,[16] and part of the oleic acid added to the nutrient solution might not have been available to the algae. It could then be speculated that oleic acid was simply adsorbed on the cell surface of the algae. However, the results show that oleic acid was well taken up by the cells, since it was rapidly incorporated into the lipids, and after a 3·5-h incubation less than 40% of the radioactivity recovered in a lipid fraction extracted as in Ref. 8 was present as free fatty acids. In the control cells, 6·8% of the label taken up was recovered in a precipitate formed during saponification of the algae. Incorporation of the label into this non-lipid fraction was inhibited by very low concentrations of the *S* isomer, less than 10^{-7} M being sufficient to observe a 50% inhibition after a 3·5-h incubation (Fig. 7). In contrast, 10^{-7} M of the *R* form yielded only 24% inhibition and 10^{-5} M was not sufficient to give 50% inhibition. The *S* isomer, the activity of which can be compared to that of metazachlor,[7] was over 500 times more active than the *R* isomer.

Many agrochemicals contain a chiral centre,[17] and the importance of this for herbicidal activity is not unique to dimethenamid. It has also been demonstrated for photosystem II herbicides, for example the *S* isomers of triazine and urea derivatives were found to bind the D1 protein and inhibit photosystem II better than the *R* isomers.[18] Interestingly, the *R* isomers of substituted triazines were more active than the *S* form in light-independent root growth inhibition in *E. crus-galli*[19] and in inducing rhizome growth in *Cyperus serotinus* Rottb.[20] Two stereoisomers were also described for the chloroacetamide metolachlor.[21] Like dimethenamid, herbicidal activity of this chloroacetamide was not

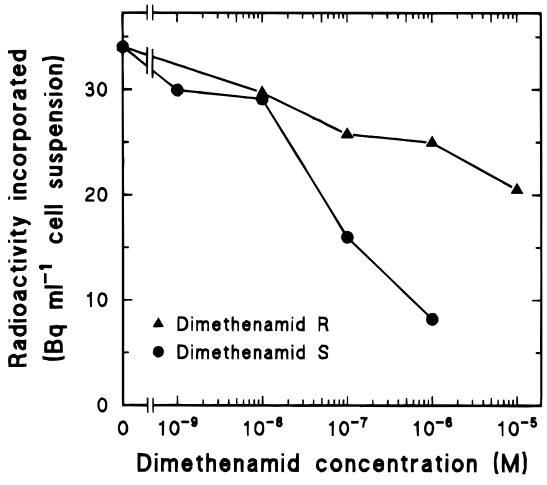


Fig. 7. Incorporation of radioactivity from oleic acid into a non-lipid fraction of *Scenedesmus acutus*. The culture (50 μg chlorophyll ml^{-1}) was incubated for 3·5 h with dimethenamid and [1-^{14}C]oleic acid (C18 : 1, 0·265 μM).

determined by the chiral axis but markedly by the configuration of the asymmetrically substituted C atom, the a*RS*,1′*S* isomer having much higher herbicidal activity than the a*RS*,1′*R* isomer, whereas the latter was found to possess higher fungicidal activity than the a*RS*,1′*S* form.[21] Up to now, no biological effect of dimethenamid has been found where the *R* isomer was more active than the *S* isomer.

The similar biological activities of dimethenamid and alachlor described in this study indicate a common mode of action for dimethenamid and chloroacetamides. The effects of chloroacetamide on various plant materials, and with different herbicide concentrations, have previously led to the conclusion that these herbicides might have multiple sites of action in the cell, possibly *via* alkylation.[4,5,22] However, a low effective concentration of dimethenamid or chloroacetamides leads to a 50% inhibition of oleic acid incorporation into the non-lipid fraction after only a few hours of incubation. The same stereospecificity relates to growth, fatty acid desaturation, acetate uptake into the cell as well as to incorporation of oleic acid. These results strongly support our conclusion that these classes of herbicide may affect a specific primary target, probably specific to lipid metabolism or the incorporation of fatty acids into non-lipid compounds. Recent data suggest that a biopolymer may be involved.[23] This target is under further investigation and the availability of the two stereoisomers of dimethenamid with a marked difference in their herbicidal activity is an important tool in our search for this target.

REFERENCES

1. Harr, J., Seckinger, K., Ummel, E. & Hargett, L. T., SAN 582 H—A new herbicide for weed control in corn and soybeans. *Brighton Crop Prot. Conf.—Weeds*, Vol. 1, 1991, pp. 87–92.

2. Chollet, R. & Harr, J., SAN 582 H: Site of uptake. *WSSA Abstracts*, **32** (1992) no. 285.
3. Harr, J., Chollet, R. & Seckinger, K., SAN 582 H: Differential activity of the isomers. *WSSA Abstracts*, **32** (1992) no. 284.
4. Fuerst, E. P., Understanding the mode of action of chloroacetamide and thiocarbamate herbicides. *Weed Technol.*, **1** (1987) 270–7.
5. LeBaron, H. M., McFarland, J. E., Simoneaux, J. B. & Ebert, E., Metolachlor. In *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 3, ed. P. C. Kearney and D. D. Kaufman. Dekker, New York, 1988, pp. 335–82.
6. Weisshaar, H., Retzlaff, G. & Böger, P., Chloroacetamide inhibition of fatty acid synthesis. *Pestic. Biochem. Physiol.*, **32** (1988) 212–16.
7. Couderchet, M. & Böger, P., Chloroacetamide-induced reduction of fatty acid desaturation. *Pestic. Biochem. Physiol.*, **45** (1993) 91–7.
8. Weisshaar, H. & Böger, P., Primary effects of chloroacetamides. *Pestic. Biochem. Physiol.*, **28** (1987) 286–93.
9. Weisshaar, H. & Böger, P., Further effects of chloroacetamides and evidence for the inhibition of acetylation of DNA-associated proteins. *Pestic. Biochem. Physiol.*, **39** (1991) 20–6.
10. Couderchet, M., Rumbolz, J., Kring, F. & Böger, P., Characteristics of a metazachlor-tolerant *Scenedesmus acutus* cell line. *Pestic. Biochem. Physiol.*, **52** (1995) 222–33.
11. Kring, F., Couderchet, M. & Böger, P., Inhibition of oleic acid incorporation into a non-lipid fraction by chloroacetamide herbicides. *Physiol. Plant.*, **95** (1995) 551–8.
12. Chollet, R., Screening of peroxidizers that cause an accumulation of photosensitive tetrapyrroles (Qualitative *Lemna* assay). In *Target Assays for Modern Herbicides and Related Phytotoxic Compounds*, ed. P. Böger and G. Sandmann. Lewis Publ. Boca Raton, Fla, USA, 1993, pp. 277–81.
13. Couderchet, M. & Böger, P., Changes in fatty acid profile induced by herbicides. In *Target Assays for Modern Herbicides and Related Phytotoxic Compounds*, ed. P. Böger and G. Sandmann. Lewis Publ., Boca Raton, Fla, USA, 1993, pp. 175–81.
14. Sinchumpasak, O., Microalgal biomass production in Thailand. In *Algae Biomass*, ed. G. Shelef and C. J. Soeder. Elsevier, North-Holland Biomedical Press, Amsterdam, 1980, pp. 115–21.
15. Kring, F. & Böger, P., Histone acetylation is not affected by chloroacetamides *in vitro*. *Z. Naturforsch.*, **49c** (1994) 309–11.
16. Mailman, D. & Rose, C., Binding and solubility of oleic acid to laboratory materials: a possible artifact. *Life Sci.*, **47** (1990) 1737–44.
17. Williams, A., Opportunities for chiral agrochemicals. *Pestic. Sci.*, **46** (1996) 3–9.
18. Gardner, G. & Sanborn, R., The role of chirality in the activity of photosystem II herbicides. *Z. Naturforsch.*, **42c** (1987) 663–9.
19. Omokawa, H. & Konnai, M., Inhibition of *Echinochloa crus-galli* var. *frumentacea* seedling root elongation by chiral 1,3,5-triazine in the dark. *Pestic. Sci.*, **35** (1992) 83–6.
20. Omokawa, H., Takeuchi, M. & Konnai, M., Rhizome induction activity of chiral 2-a-methylbenzylamino-4-alkylamino-6-chloro-1,3,5-triazines in *Cyperus serotinus* Rottb. *Pestic. Sci.*, **35** (1992) 87–90.
21. Moser, H., Rihs, G. & Sauter, H., Der Einfluß von Atropisomerie und chiralem Zentrum auf die biologische Aktivität des Metolachlor. *Z. Naturforsch.*, **37b** (1982) 451–62.
22. Leavitt, J. R. C. & Penner, D., *In-vitro* conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788. *J. Agric. Food Chem.*, **3** (1979) 533–6.
23. Couderchet, M., Schmalfuß J. & Böger, P., Incorporation of oleic acid into sporopollenin and its inhibition by the chloroacetamide herbicide metazachlor. *Pestic. Biochem. Physiol.* **55** (1996) 189–99.